CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

FORCHLORFENURON

Chemical Code # 5557, **Tolerance #** 52650
SB 950 # NA

July 9, 2004

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, no adverse effects
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Study not submitted, not required at this time.

Toxicology one-liners are attached.

All record numbers through 211661 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T040709
Revised by T. Moore, 7/9/04

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 52650-049; 167418; ACombined 24-Month Dietary Toxicity/Oncogenicity Study in Rats with SKW 20010" (Mertens, J., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-171004, 8/22/96). Seventy rats/sex/dose were offered feed containing the following concentrations of SKW 20010 (Batch #92120012, purity: 98.5%) for up to 2 years: 0, 150, 2000 and 7500 ppm. The corresponding values for mean subject product consumption were (M) 0, 7, 93, 352 mg/kg/day; (F) 0, 9, 122, 518 mg/kg/day. Mortality was not increased by the test article. Neither did the compound induce any increase in palpable masses (by physical exam) or neoplasms (by pathological exam). Mean bodyweights of animals fed 7500 ppm were significantly lower than controls following 1 week of test article feeding and continuing to the study=s end (males 14%, females 31% lower at week 103). Females fed 2000 ppm were also significantly lower (11.2% by week 103), but males were not. Food consumption correlated well with decreased bodyweights, being significantly lower than controls for a total of 43 weeks in females fed 7500 ppm, followed by males fed 7500 ppm (lower for 29 weeks) and females fed 2000 ppm (lower for 23 weeks). Abnormal clinical signs were limited to increased urogenital and ventral abdominal matting in both sexes; in males the number of incidents was increased at both 2000 ppm and 7500 ppm while in females only the animals fed the higher dose were consistently affected. Pathological exams suggested a possible reason for these clinical signs: an increased incidence of irregularities in the kidneys of animals fed the test article (other organs were normal). Gross pathological changes included white areas, cysts and depressed areas in both males (2000 and 7500 ppm) and females (7500 ppm). Correspondingly, the kidneys were the only organ to show microscopic changes which included suppurative inflammation, tubular dilatation and interstitial nephritis, in both sexes at 7500 ppm and less frequently in males at 2000 ppm. Clinical pathology (hematology, serum chemistry, urinalysis) identified the following significant compound-induced alterations in both sexes fed 7500 ppm, and the weeks of maximal change: increased white cells (males 49% at week 103, females 32% at week 51), increased platelets (males 18% at week 103, females 11% at week 25), increased lymphocytes (males 26% at week 25, females 29% at week 51), increased serum phosphorus (males 14% at week 25, females 19% at week 51). **No adverse effects indicated. Chronic NOEL (M/F)** 150 ppm (M: 7 mg/kg/day, F: 9 mg/kg/day; based on the increased incidence of gross pathologic and microscopic changes to the kidneys of males fed 2000 ppm and decreased mean bodyweights of females fed 2000 ppm); **Study Acceptable** (Vidair 7/30/99).

CHRONIC TOXICITY, RAT

See Combined, Rat.

CHRONIC TOXICITY, DOG

** 0061; 211642; “A One Year Dietary Toxicity Study of CPPU in Dogs”; (J.J.W.M. Mertens; WIL Research Laboratories, Inc., Ashland, OH; Project No. WIL-296001; 12/8/98); Four beagle dogs/sex/group received 0, 150, 3000 or 7500 ppm of CPPU (Forchlorfenuron Technical) (lot no. 97030010, purity: 96.8%) in the diet for 52 weeks. One male in the 7500 ppm group died during the 32nd week. The death was attributed to diffuse pleuritis and was not considered to be treatment-related. Mean body weight gain was reduced for the 7500 ppm females over the course of the study. Mean food consumption was lower for the males in the 3000 ppm treatment group and for both sexes in the 7500 ppm treatment group (NS). No treatment-related clinical signs were noted during the study other than one female in the 7500 ppm group which was thin in appearance. In the hematology evaluation, the mean red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin of the 7500 ppm females were lower than those of the controls at 12, 25 and/or 51 weeks ($p<0.05$ or 0.01). In the clinical chemistry evaluation, the mean serum cholesterol concentrations for both sexes in the 3000 and 7500 ppm groups were increased over that of the controls at 12, 25 and/or 51 weeks ($p<0.05$ or $p<0.01$). In the necropsy examination, the relative liver and kidney weights of

the 7500 ppm females were greater than those of the controls ($p<0.01$). However, no treatment-related lesions were noted in the histopathological examination. **No adverse effect indicated. Chronic NOEL:** 150 ppm (M and F: 5 mg/kg/day) (based upon the increased concentration of cholesterol in the serum for both sexes in the 3000 ppm treatment group); **Study acceptable.** (Moore, 6/29/04)

ONCOGENICITY, RAT

See Combined, Rat.

ONCOGENICITY, MOUSE

** 0062; 211643; "A 18-Month Dietary Carcinogenicity Study of CPPU in Mice"; (J.J.W.M. Mertens; WIL Research Laboratories, Inc., Ashland, OH; Study No. WIL-296003; 1/6/00); Sixty CD-1 mice/sex/group received 0, 10 or 1000 mg/kg/day of CPPU (Forchlorfenuron Technical) (lot no. 97030010, purity: 96.9%) in the diet for 18 months. The content of the dietary preparations was adjusted as the food consumption and body weights of the treated animals changed during the study. There was no treatment-related effect upon survival. The mean body weights of both sexes in the 1000 mg/kg group were less than those of the controls at various times over the course of the study ($p<0.05$ or 0.01). No treatment-related effects were noted in the differential white blood cell count. The apparent target organ was the kidney. The mean absolute and relative kidney weights for both sexes in the 1000 mg/kg group were greater than those of the controls ($p<0.01$). In the histopathological examination, a greater incidence of renal tubular dilatation was noted for both the 10 and 1000 mg/kg females and for the 1000 mg/kg males ((M) 0: 1/60 vs. 10: 2/60, 1000: 7/60, (F) 0: 2/59 vs. 10: 7/60, 1000: 13/60). Although the relative liver and testes weights for the 1000 mg/kg males were greater than those of the controls, no treatment-related lesions were evident in the microscopic examination. **No adverse effect was evident. Chronic NOEL (M/F):** 10 mg/kg/day (based upon increased absolute and relative kidney weights and kidney lesions); **Carcinogenicity not evident**. **Study acceptable.** (Moore, 7/2/04)

REPRODUCTION, RAT

** 52650-048; 167417; ADietary Two-Generation Reproduction Study of SKW 20010 in Rats@ (Nemec, M.D., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-171002, 3/27/97). Thirty rats/sex/dose were offered feed containing the following concentrations of SKW 20010 (Batch #92120012, purity: 98.5%) for two generations (F0 and F1): 0, 150, 2000 and 7500 ppm. F0 parents were fed the test article for at least 70 days prior to mating, throughout breeding, and until necropsy. The F0 parents were bred twice, producing two litters (F1a, F1b) which were exposed to the test article in utero and during nursing, through lactation day 21 (the time of necropsy). Selected F1b pups (the F1 generation) were offered the test article beginning on day 22 post partum and thereafter, as described above for their F0 parents. These animals were also bred twice, producing two litters (F2a, F2b) which were exposed to the test article as described above for the F1a and F1b litters. There was no compound-related mortality. Mean bodyweights of F0 and F1 parents fed 7500 ppm were significantly lower than controls throughout the study periods (F0 parents: 82.4 to 94.8% of controls, $p\leq0.05$; F1 parents: 28.9 to 73.4% of control, $p\leq0.05$). F1 parents fed 2000 ppm, but not F0 parents, were also significantly lower than controls for the majority of weeks (86.5 to 94.5% of control, $p\leq0.05$). Relative organ weights, but not absolute organ weights, were increased in the 2000 and 7500 ppm groups, indicating that the test article effect on organ weight was most likely indirect. However, kidney abnormalities were noted at 7500 ppm, both by gross pathological and microscopic examination. These irregularities included white areas, cysts and other abnormalities by gross pathology, and suppurative inflammation, cysts, suppurative pyelonephritis and interstitial nephritis by histopathology. Reproductive performance was not affected. The test article showed developmental effects on both pup viability and bodyweight. Pup viability was significantly decreased (average 4.7% lower than controls) in litters F1a, F1b and F2a produced by females fed 7500 ppm. Necropsy of these found dead pups revealed pups with dark red contents of the stomach and/or pups classified as emaciated, both only in the 7500 ppm group. Mean pup bodyweights were significantly lower (32 to 40% of control, $p\leq0.05$, by lactation day 21) for all 4 litter groups from mothers fed 7500 ppm. At 2000 ppm, 3 litter groups showed smaller but significant decreases (89 to 94% of control,

p≤0.05, by day 21). **No adverse effects indicated. Parental NOEL:** 150 ppm (based on lower mean bodyweights for F1 males and females fed 2000 and 7500 ppm); **Reproductive NOEL:** 7500 ppm (based on the absence of effects on reproduction at the highest dose tested); **Developmental NOEL:** 150 ppm (based on lower mean pup bodyweights from litters of females fed 2000 and 7500 ppm); **Study Acceptable.** (Vidair 7/26/99).

TERATOLOGY, RAT

** 52650-039; 167407; AA Teratology Study in Rats with CN-11-3183" (Koehler, S.V., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-15207A, 6/24/86). Test article CN-11-3183 (Lot #F50002, 98.9% purity) was administered daily by oral gavage to pregnant rats on days 6 through 15 of gestation. Twenty five rats/dose were given the following: 0 (corn oil), 100, 200, or 400 mg/kg/day. There was a single mortality on day 16 in the highest dose group. In-life clinical signs included: a dose-dependent increase in the number of incidents of hair loss; 200 mg/kg/day-scabbing on the abdomen and hind limb, yellow urogenital staining; 400 mg/kg/day-lethargy, ataxia, scabbing on the abdomen and hind limb, transparent eyes, yellow/red urogenital staining/discharge. The mean increase in bodyweights between days 0 and 20 was lower for the highest dose only: 109 grams for 400 mg/kg/day versus 130 grams in controls (p<0.01). Most of this effect was due to decreased weight gain between days 6 and 16. There were no effects on intrauterine fetal survival or fetal sex ratios. The only statistically significant decrease was in mean fetal weight: from 3.5 grams in the control to 3.1 grams in the 400 mg/kg/day group (p<0.05). Abnormalities revealed at necropsy included: 100 mg/kg/day-fused placenta, dilated pelvis of the kidney; 200 mg/kg/day-none; 400 mg/kg/day-the single mortality exhibited blood in the nasal, buccal, and urogenital areas, dark brown contents of the intestines, 3 early and 14 late resorptions, and hemorrhage of the uterus. The hemorrhage of the uterus was considered to have been due to the test compound and was the cause of death. None of the fetal malformations showed a dose-response or were significantly elevated in the treated animals versus controls. They included: 100 mg/kg/day-diaphragmatic hernia, retroesophageal aortic arch; 200 mg/kg/day-none; 400 mg/kg/day-exencephaly, lobular agenesis of the lungs. Among these fetal malformations, only exencephaly was represented in the historical control data (838 litters examined); its incidence in the instant study fell within the range of historical controls. The numbers of fetal variations were not significantly different in treated animals versus controls. However, two abnormalities were both above control levels and above the range of historical controls: unossified sternebra (E) #1, #2, #3 and/or #4 and reduced ossification of the 13th rib(s), both in the highest dose group. **Maternal NOEL =** 200 mg/kg/day (based on decreased bodyweight gain for animals fed 400 mg/kg/day). **Developmental NOEL =** 200 mg/kg/day (based on a lower mean fetal weight for animals in the 400 mg/kg/day group). **Study acceptable** (Vidair 8/5/99).

The homogeneity of the dosing preparations in the rat teratology study was analyzed after the fact due to the failure to perform this analysis at the time of the study. These data are contained in vol. 52650-0069, rec. no. 211650.

The daily observation tables for the does in the rabbit teratology study have been submitted in vol. 52650-0079, rec. no. 211661.

52650-038; 167406; AA Range-Finding Teratology Study in Rats with CN-11-3183" (Young, D.L., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-15198, 12/9/85). Test article CN-11-3183 (Lot #RA22901, purity: 98.9%) was administered daily by oral gavage to pregnant rats on days 6 through 15 of gestation. Five rats/dose were given the following: 0 (corn oil, 50, 125, 250, 500, or 1000 mg/kg/day. One rat given 500 mg/kg/day died on day 15 of gestation and one rat given 1000 died on each of days 13, 14, 15, and 19. There was no effect on bodyweights for animals given up to 250 mg/kg/day. Other than urogenital matting and severe bloody vaginal discharge (500, 1000 mg/kg/day), all other in-life signs occurred only at the highest dose and included lethargy, dehydration, dried red or yellow material about the mouth or eyes, wet yellow matting of the abdomen, dried blood on the forepaws, rough hair coat, soft stool, and mucoid feces with blood. Uterine exams identified effects by the highest dose only. The lone survivor had

no viable fetuses and exhibited total resorption. Abnormalities noted at necropsy were: 0 mg/kg/day, none; 50 mg/kg/day, (1/5) congested lungs, kidney pitted; 125 mg/kg/day, (1/5) hydronephrosis of the left kidney; 250 mg/kg/day, (2/5) congested lungs; 500 mg/kg/day, (1 mortality) hemorrhage of the brain, congested kidneys, enlarged adrenals, ulcerations of the stomach, hemorrhage of the intestines, urinary bladder contained dark solid material; 1000 mg/kg/day, (4 mortalities) enlarged and/or pale adrenals, hemorrhage or congestion of the brain, stomach ulcers, intestinal abnormalities, congested or pale kidneys, dark red or congested lungs, dark red and firm thymus. **No adverse effects. Supplemental Study.** (Vidair 7/16/99).

TERATOLOGY, RABBIT

52650-041; 167409; AA Teratology Study in Rabbits with CN-11-3183" (Young, D.L., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-15208, 5/14/86). Test article CN-11-3183 (Lot #F50002, 99.9% purity) was administered daily by oral gavage to pregnant rabbits on days 6 through 18 of gestation. Eighteen rabbits per dose were given the following: 0 (0.5% aqueous methylcellulose), 25, 50, or 100 mg/kg/day. The 2 mortalities (0, 100 mg/kg/day) on gestation days 5 and 16, respectively, and 3 abortions (25, 50 mg/kg/day) were not considered compound related. The following clinical signs were increased in treated animals: decreased defecation and urination, soft stool, anogenital matting. Mean bodyweights for the lowest dose group (25 mg/kg/day) were never significantly different from controls and increased throughout. In contrast, animals in the 50 and 100 mg/kg/day groups were decreased in mean bodyweight from gestation days 12 to 24, by which time they were significantly lower than controls (6% and 9%, respectively, $p<0.05$). By day 29 these animals recovered so that their mean bodyweights were no longer significantly different from those of controls. Upon Cesarean section at gestation day 29, no effects were seen on fetal sex ratios, fetal viability, resorptions, post-implantation loss, implantation sites, corpora lutea, or fetal weight. Fetal morphology was probably not affected by the test article since the number of fetuses with malformations actually dropped at the highest dose relative to controls (1 versus 3), and the total number of variations was similar in controls and treated animals. All types of malformations and variations were within historical control values and/or exhibited no clear dose-response. Thus, the highest dose tested (100 mg/kg/day) was not considered teratogenic in this strain of rabbit. **Maternal NOEL = 25 mg/kg/day (based on decreased mean bodyweights from gestation days 12 to 24 for animals fed 50 mg/kg/day). **Developmental NOEL =** 100 mg/kg/day (based on an absence of effects by 100 mg/kg/day on fetal development). **Study acceptable** (Vidair 8/5/99).

The homogeneity of the dosing preparations in the rabbit teratology study was analyzed after the fact due to the failure to perform this analysis at the time of the study. These data are contained in vol. 52650-0069, rec. no. 211650.

The daily observation tables for the does in the rabbit teratology study have been submitted in vol. 52650-0078, rec. no. 211660.

52650-040; 167408; AA Range-Finding Teratology Study in Rabbits with CN-11-3183" (Young, D.L., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-15199, 12/9/85). Test article CN-11-3183 (RA 22901, 98.9% purity) was administered daily by oral gavage to pregnant rabbits on days 6-18 of gestation. Five rabbits per dose were given the following: 0 (0.5% aqueous methylcellulose), 50, 125, 250, 500, or 1000 mg/kg/day. Mortality was 2/5 at 500 (both dying on gestation day 18) and 4/5 at 1000 mg/kg/day (on gestation days 15, 17, 18, 20). At necropsy the following were noted in these animals: liver pale and soft/biliary stasis; kidneys pale and soft/congested; trachea reddened; intestine congested/loss of epithelium/hemorrhagic; stomach ulcerations/loss of epithelium/large opening near cardiac valve; lungs dark red and firm/dark grey; uterus severe hemorrhage. Causes of death in 2 animals were determined to be aspiration pneumonia or overt toxicity. One animal from both the 250 and 125 groups aborted on days 21 and 24, respectively. At necropsy the following were noted in these animals; liver pale and soft; kidneys pale and soft; lymph nodes enlarged with greenish fluid; loss of epithelium in cecum; clear fluid in the hydrothorax; lungs congested/white foci. Dose-related bodyweight loss was reported over gestation days 6 to 18 for doses of 125 mg/kg and higher. There were no pregnant females

alive in the 1000 mg/kg/day group at 30 days. Clinical signs in treated animals included hair loss, diarrhea, brown anogenital staining, decreased urination, decreased defecation and red material on cage paper. Uterine examinations on day 29 showed changes in the following at 250 mg/kg/day and above: decreased mean number of viable fetuses, increased mean number of early resorptions, and increased mean post-implantation loss. There were no dead fetuses and no significant effects on implantation sites or corpora lutea; however, there were no pregnant animals alive for the uterine exam on day 29 from the highest dose group. **Maternal NOEL < 50 mg/kg/day** (based upon clinical signs). **Developmental NOEL not determined. Supplemental Study.** (Vidair, 7/16/99).

GENE MUTATION

**** 52650-052**; 167422; ASalmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)@ (Lawlor, T.E., Microbiological Associates, Bethesda, MD, Project ID No. T4015.501, 7/22/85). *S. typhimurium* tester strains TA98, TA1537, TA1538 (reverted to histidine prototrophy by frame shift mutation), TA100, and TA1535 (reverted by base substitution) were exposed to test article CN-11-3183 (Lot #F50002, 98.9% purity) for 48 hours at 37^{O}C, with or without activation by an S9 microsomal fraction. Doses of test article ranged from 10 to 1000 ug/plate with activation, and from 2 to 200 ug/plate without activation. Each dose was tested for reversion of histidine auxotrophy in a single trial with 3 replicate plates. From among the 5 tester strains, only TA1535 showed an induction of reversion by the test article: a 2.2-fold induction by 100 ug/plate only in the absence of activation. A dose-response was also indicated. Positive controls were functional. These data suggest that the CN-11-3183 induces mutations via base substitution. **Possible adverse effect indicated. Study acceptable** (Vidair 8/4/99).

52650-053; 167423; ASalmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)@ (Lawlor, T.E., Microbiological Associates, Bethesda, MD, Laboratory Project ID # T4278.501004, 9/26/85). *S. typhimurium* tester strain TA1535 (reverted to histidine prototrophy by a base substitution) was exposed to test article CN-11-3183 (Lot #F50002, 98.9% purity) for 48 hours at 37^{o} C. Doses of test article ranged from 10 to 1000 ug/plate (with S9 activation) and from 2 to 200 ug/plate (without activation). Each dose was tested in triplicate (single trial) for induction of reversion to histidine prototrophy. In the absence of activation only, the test article at 50 ug/plate produced a 2.6-fold increase in the number of revertants Positive controls were functional. These data suggest that CN-11-3183 induces mutations by base substitution. **Possible adverse effect indicated. Study supplemental** (Vidair 8/4/99).

CHROMOSOME EFFECTS

** 52650-050; 167420; "Chromosome Aberrations Assay in Chinese Hamster Ovary (CHO) Cells" (Putman, D.L., Microbiological Associates, Inc., Bethesda, MD, Lab-Project # T4015-337, 7/25/85). Chinese hamster ovary cells (CHO-K_1) were exposed to 10, 20, 40 or 80 μg/ml of CN-11-3183 (Lot #F50002, 98.9% purity), with or without activation by fraction S9 (isolated from Aroclor 1254-induced rat liver). In the nonactivation protocol, cells were exposed to the test article for 10 hours, the last 2-3 hours in the presence of colcemid to facilitate the accumulation of metaphase cells. In the activation protocol, cells were exposed to the test article for 2 hours in the presence of the S9 fraction, followed by washing and incubation in fresh medium for 8 hours, the last 2-3 hours in the presence of colcemid. Single trial, duplicate cultures, 50 cells/culture were scored for a total of 100 cells/dose. The test article did not cause a significant increase in chromosome aberrations, measured as either the number of cells with structural aberrations or the number of structural aberrations per cell. There was also no effect on the fraction of cells with abnormal chromosome numbers. Activation had no effect. In contrast, positive controls triethylenemelamine (nonactivated protocol) and cyclophosphamide (activated protocol) induced significant increases in the number of structural aberrations per cell. **No adverse effects indicated. Study Acceptable** (Vidair 8/2/99).

DNA DAMAGE

** 52650-051; 167421; AUnscheduled DNA Synthesis in Rat Primary Hepatocytes@ (Curren, R.D., Microbiological Associates, Bethesda, MD, Project ID No. T4015.380, 7/26/85). Primary

cultures of rat liver cells were incubated for 18-20 hours with 10 uCi/ml of ^{3}H-thymidine and the following concentrations of test article CN-11-3183 (Lot #50002, purity: 98.9%): 0, 0.1, 0.3, 1.0, 3.0, 10, 30 or 60 ug/ml. A vehicle only control (acetone) and positive control (DMBA: 7,12-dimethylbenzanthracene: 3, 10 ug/ml) were included. Cells were processed for autoradiography and silver grains over individual nuclei (25/replicate x 3) were counted. Doses up to and including 10 ug/ml caused small decreases in cell survival relative to the vehicle control, without inducing any significant increase in unscheduled DNA synthesis. In contrast, both doses of DMBA caused a greater than 60-fold increase in the average number of grains per nucleus, while at the same time lowering relative cell survival by 56% (3 ug/ml) and 76% (10 ug/ml). 60 ug/ml or 30 ug/ml of test article caused so much toxicity (relative cell survival=0%) that the assay for unscheduled DNA synthesis could not be performed. The data suggest that the test article did not cause a significant induction of unscheduled DNA synthesis. **No adverse effects indicated. Study acceptable** (Vidair 8/2/99).

NEUROTOXICITY

Study not submitted. Not required at this time.

SUBCHRONIC TOXICITY

Rat 28-day feeding study

52650-042; 167410; A28-Day Dietary Range-finding Study in Rats with CN-11-3183" (Tasker, E.J., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-15200, 4/9/86). Test article CN-11-3183 (98.9% pure; RA 22901) was offered ad libitum to 5 male and 5 female rats for 28 days at each of the following feed concentrations: 0, 300, 1000, 3000, 10000 ppm. There were no deaths or abnormal clinical signs. All mean bodyweights increased weekly except for rats fed 10000 ppm. Measurement of weekly bodyweight gains revealed the only significant difference between the group fed 3000 ppm and controls: that of approximate 61% and 85% lower weight gains for males and females during week 3, respectively. Food consumption, measured as grams/animal/day or grams/kg/day, was decreased relative to controls only at the highest dose, in both sexes. Lastly, necropsy was normal in all but 2 females: one (300 ppm) with a dilated renal pelvis and one (3000 ppm) with dark red lungs. **NOEL (M/F) = 1000 ppm** (M: 76.8 to 115.7 mg/kg, F: 83.7 to 108.6 mg/kg; based upon reduced bodyweight gain). **No adverse effects indicated. Supplemental Study.** (Vidair 7/16/99).

Rat 90-day feeding study

52650-043; 167411; A90-Day Dietary Study in Rats with CN-11-3183" (Tasker, E.J., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-15209, 1/12/87). Test article CN-11-3183 (98.9% purity, Lot #F0002) was offered to 20 male and 20 female rats ad libitum for 90 days at each of the following feed concentrations: 0, 200, 1000 and 5000 ppm. One animal from each dose group (including control) died or was sacrificed moribund. Pathological exams identified different causes of death for each, none of which was considered compound-related. In-life signs were predominantly hair loss, with none exhibiting a dose-response. The mean bodyweights of females fed 5000 ppm were significantly lower than controls, ranging between 6 and 10% lower for weeks 3 through 13 (with the exception of week 6). Food consumption (grams/kg/day) could not account for this decreased weight gain, since it was not significantly decreased. Tests in hematology, serum chemistry, urinalysis and ophthalmology identified a few statistically significant alterations in animals fed the test article; however, these changes were sporadic and within the normal historical ranges for this strain of rat, and therefore were not considered compound-related. Pathological exams of scheduled deaths detected an increase in dilated renal pelvis of the kidneys among treated females. This abnormality may have shown a dose-response (incidence of 1, 1, 3, 5 for 0, 200, 1000, 5000 ppm); however, more animals are needed to determine if the dose-response is real. Liver weight in the 5000 ppm dosage group was the only parameter exhibiting a consistent change relative to controls: free organ weights-(M) a 13-15% increase at 1000-5000 ppm, (F) a 10% increase at 5000 ppm; organ weights/final body weights-(M) an 11% increase at 5000 ppm, (F) a 21% increase at 5000 ppm; organ weights/final brain weights-(M) a 15-17% increase at 1000-5000 ppm, (F) a 13% increase at 5000 ppm. Microscopic examination

of liver sections provided no clear cellular basis for the increased liver weights. A decrease in weight of the adrenals was primarily observed at 200 ppm. Other statistically significant changes in organ weights were measured in the heart and kidneys when normalization was to final body weight; however, these alterations were not observed when normalization was to brain weight or when free organ weights were used. **No adverse effects indicated. NOEL (M/F)** 1000 ppm based on decreased mean bodyweights for females and increased liver weights for both males and females at 5000 ppm (the increase in liver weights at 5000 ppm was significant by all methods of organ weight normalization). **Study acceptable** (Vidair 7/13/99).

Dog 28-day feeding study

52650-044; 167412; A28-Day Dietary Study in Dogs with CN-11-3183" (Laveglia, J., WIL Research Laboratory, Ashland, OH, Project ID No. WIL-15201, 5/23/86). One male and one female Beagle dog/dose were offered feed containing each of the following concentrations of CN-11-3183 (RA 22901, purity: 98.9%): 0, 150, 500, 2500, 7500 ppm. Food containing the test article was offered for 2 hours/day for 10 days, followed by a 5 day interval when feed without test article was mistakenly used, followed again by a 42 day interval of daily compound presentation in the feed. Weekly samples of feed were analyzed chemically to ensure that proper dosage levels were maintained. No animals died during the study and there were no abnormal clinical signs. Similarly, necropsy at study termination revealed no abnormalities common to the different dosage levels. Despite the use of only one male and one female per dose, the data suggest that the subject product did affect bodyweights and food intake. The male fed 7500 ppm exhibited decreased weight gain relative to the control, not exceeding its starting weight until week 5. Females were more sensitive; animals fed 2500 or 7500 ppm suffered both an early growth delay and a late weight loss. Smaller but similar effects may have been seen in the females fed the two lower doses. Food intake on a grams/animal/day basis was reduced for males at 7500 pm and for females at 7500 and 2500 ppm. When food consumption was normalized to animal weight (grams/kg/day), the compound=s effect was only evident at the highest dose of 7500 ppm, again for both sexes. **No adverse effects indicated. Supplemental study.** (Vidair 7/19/99).

Dog 90-day feeding study

52650-045; 167413; A13-Week (90-day) Oral Range-Finding Toxicity Study in Dogs with SKW 20010" (Shour, M.H., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-171003, 3/10/93). Two male and two female Beagle dogs/dose were offered feed containing each of the following concentrations of SKW 20010 (purity: 99.4%): 0, 7500, 10000 ppm. The food was offered for one hour/day for 91 consecutive days. Samples of feed taken at weeks 0, 1, 6 and 13 were analyzed chemically to ensure that proper dosage levels were maintained. Consumption of test article for the 7500 and 10000 ppm dosage levels was 173 and 212 mg/kg/day for males and 208 and 232 mg/kg/day for females (means of the 13 weeks). There was no animal mortality. The only compound-related abnormal clinical sign was decreased defecation in all animals fed 10000 ppm and in one male fed 7500 ppm. Both males and females lost weight for the first 3 weeks at the highest dose (mean bodyweights); the 2 males gained weight thereafter while the females remained essentially constant. At the 7500 ppm level the males= weights remained fairly constant, while the females gained weight (about 33% of the control group=s weight gain) after a 2 week lag. Decreased food consumption probably accounted for some of these effects, since it was sporadically lower for both males and females in both dosage groups. Hematology tests detected elevated white cells in males at 7500 and 10000 ppm which was primarily due to an increase in segmented neutrophils. Males also showed elevated reticulocytes for the higher dose. Females exhibited decreased red cells and decreased hematocrit at the higher dose. Although platelet counts were elevated in treated males and females, the changes were not considered compound-related since the values fell within the ranges of historical controls. Leukocyte counts and serum chemistry did not reveal any changes in treated animals which were outside the range of historical controls except for elevated cholesterol in females at 7500 and 10000 ppm (males were also elevated but within historical controls) and elevated total protein in females at 10000 ppm (only the change in protein was statistically significant). The gross pathological exam detected no abnormalities common to the 2 animals in each dosage group. Microscopic examination of fixed tissue was also negative. Lastly, mean liver weights normalized to final

bodyweights were elevated for both sexes at both doses; however, free liver weights were not elevated, indicating that the increase in normalized liver weight was due to decreased animal bodyweight. The absence of histopathologic lesions in the liver supported this conclusion. **No adverse effects indicated. Supplemental Study** (Vidair 7/16/99).

52650-046; 167415; A90-Day Dietary Study in Dogs with CN-11-3183" (Laveglia, J., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-15210, 1/14/87). Four male and four female Beagle dogs/dose were offered feed containing each of the following concentrations of CN-11-3183 (KT-30, Lot #50002, 99.9% purity): 0, 50, 500, 5000 ppm. The food was offered for 2 hours/day for 90 consecutive days. Samples of feed from weeks 1, 2, 3, 4, 8 and 12 were analyzed chemically to ensure that proper dosage levels were maintained. Calculated mean test compound consumption for the four dosage levels, in ascending concentration, were 0, 1.8, 18.0 and 176 mg/kg/day. No animals died during the study. Abnormal in-life clinical signs were never detected in more than one animal per dosage level and were short in duration; therefore, they were not considered to be compound-related. Mean bodyweights of treated animals were not significantly different from controls. Tests in hematology, serum chemistry and urinalysis detected few statistically significant differences between treated animals and controls. MCH was significantly lower at 90 days for females fed 5000 ppm; however, lack of any effect in males or in other red cell parameters suggested that the test compound was not the cause. The alterations in SGOT activity, while significant, fit no pattern consistent with any dose-response. Only cholesterol levels in both males and females were consistently higher than controls at 5000 ppm, at both 45 and 90 days (although the increases at 90 days were not statistically significant). Pathological exams identified two alterations which each occurred in treated animals but not in controls: nodules in the duodenum and a dark blue or enlarged spleen. However, there was no dose-response for either abnormality and microscopic examination of the tissues did not support a compound-related etiology. Lastly, whether organ weights were normalized to final bodyweight or brain weight, there were no significant effects in treated animals. **No adverse effects indicated. NOEL (M/F)** 500 ppm (based on elevated serum cholesterol levels at 45 and 90 days for animals fed 5000 ppm, with only the 45 day levels being significantly above controls). **Study Acceptable.** (Vidair 7/19/99).

Mouse 90-day feeding study

52650-047; 167416; A13 Week Dietary Range-Finding Study of SKW 20010 in Mice@ (Lambing, C.A., WIL Research Laboratories, Ashland, OH, Project ID No. WIL-171007, 5/11/95). Ten male and ten female mice/dose were offered food containing each of the following concentrations of SKW 20010 (Batch #92120012, 100% purity): 0, 900, 1800, 3500 or 7000 ppm. Food containing the test article was offered ad libitum for 90 days. Mean consumption of test compound at the 5 dosage levels in ascending order was males/females 162/206, 332/411, 609/788 and 1288/1683 mg/kg/day. There was no mortality and clinical signs were limited to a higher incidence of scabbing and reddening of the ears of treated males; however, there was no dose-response for this clinical sign and it was not observed in females. Only animals fed 7000 ppm had mean bodyweights which were lower than controls. This was true for both males and females, starting at 3 weeks. However, only in the case of males during the ninth week was the difference statistically significant. Food consumption was significantly lower for animals fed the highest dose, for a total of 4 weeks in the case of males and for one week in the case of females. At 3500 ppm both sexes suffered a single week with significantly lower food consumption. Tests in clinical pathology at 90 days revealed no convincing compound-related effects. Platelets were elevated for all males fed the test compound, but these increases were not statistically significant, showed no dose-response, and were absent from treated females. A similar pattern was noted for elevated urea nitrogen in treated males with one difference: the increase at 900 ppm was statistically significant. Bilirubin was elevated for both males and females at 7000 and 3500 ppm; however, only the increases at 7000 ppm for females and 3500 ppm for males were statistically significant. At necropsy, 4 males and 4 females were noted as having gray lacrimal glands, compared to none in controls. However, there was no dose-response for this endpoint. Free organ weights were not significantly different for treated versus control animals. In contrast, when organ weights were normalized to final bodyweights, significant increases in liver weights were

identified in males at 3500 and 7000 ppm and in females at 7000 ppm. Normalized liver weights were also elevated in males at the two lower doses (though not significantly), perhaps suggesting a dose-response. However, since free liver weights were not significantly different from controls and microscopic examination of stained liver sections were negative, it is doubtful that the test compound caused liver enlargement. Lastly, the normalized kidney weights of females fed the highest dose were significantly elevated compared to controls, and microscopic examination of that organ revealed an increased incidence of infiltration of lymphocytes into the interstitium and pelvis (true for both males and females). However, since these infiltration events were almost always graded as minimal, and free kidney weights were not significantly elevated compared to controls, the case for a compound-related effect on the kidneys is weak. **No adverse effects indicated. Supplemental study** (Vidair 7/20/99).

METABOLISM STUDIES

Metabolism, Rat

52650-0063; 211644; "An Oral (Gavage) Metabolism Study with ^{14}C-CPPU in Rats"; (D.W. Sved; WIL Research Laboratories, Inc., Ashland, OH; Study No. WIL-296004; 11/14/02); In both the main study and the biliary excretion study, at least 4 Sprague-Dawley rats/sex were dosed orally by gavage with 100 mg/kg of CPPU-UL-phenyl-^{14}C (lot no. 000223; radiochemical purity: 99.16%; specific activity: 28.04 mCi/mmol) fortified with unlabeled Forchlorfenuron technical (lot no. 97030010, purity: 98.2%). In the main study, urine, feces and air samples were collected periodically for 7 days post-dose. In the biliary excretion study, bile samples were collected periodically via the bile duct cannula up to 72 hours post-dose. The primary route of excretion was in the urine ((M) urine: 79%, feces: 16%, (F) urine: 68%, feces: 28%). During the 1st 24 hours post-dose, 82% of the radiolabel was recovered from the males and 66% from the females. Less than 0.1% of the administered dose was recovered in the air. The excretory half-lives ranged from 13 to 16 hours for both sexes for both the urine and feces. Recovery in the tissues at 7 days post-dose represented less than 1% of the administered dose. In the biliary excretion study, 23 and 20% of the administered radiolabel were recovered in the bile from the males and females, respectively. However, the absorption kinetics could not be readily assessed because no urine or feces samples were collected simultaneously from these study animals. The primarily metabolite recovered in the urine was CPPU-sulfate with substitution on the phenyl ring. It represented 84 and 57% of the administered dose for the males and females, respectively. Other metabolites were products of phenyl ring hydroxylations as well. Hydroxyl-CPPU was the predominant metabolite recovered from the feces with 11 and 18% of the administered dose recovered from the males and females, respectively. **Study acceptable.** (Moore, 7/8/04)